results suggest that the function of cyclin I as an intrinsically disordered protein might regulate the activity of p21, another intrinsically disordered protein. This protein-protein interaction may rescue the cell from the apoptosis pathway.

3221-Pos Board B82

A Quest for Small Molecule Inhibitors of the Cell Cycle Regulator, P27

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Hearing loss is primarily caused by alteration of inner ear hair cells (HC). Humans and other mammals cannot spontaneously regenerate damaged hair cells. However, chicken, fish and amphibians have the ability to regenerate the damaged sensory HCs and recover their hearing.

In mice, HC and SC progenitors exit the cell cycle between embryonic days 12 and 14 and do not proliferate thereafter. This stage of development correlates very well with the expression of the cell cycle inhibitor, p27Kip1 (p27), in the organ of Corti, suggesting that p27 plays a role in terminal differentiation in this tissue. This hypothesis is supported by studies in which inactivation of p27 in mouse postnatal SCs or HCs has led to cell proliferation and HC regeneration, revealing the potential of p27 small molecule inhibitors (therapeutic drugs) for regeneration of functional HCs in mammalian vertebrates.

Protein p27 is an intrinsically disordered protein (IDP) that inhibits the enzymatic catalytic activity of nuclear Cdk2/cyclin E (and A) complexes, blocking progression from G1 to S phase of the cell cycle and accounting for the ability of p27 to enforce cell cycle arrest.

We hypothesize that small molecules which bind specifically and tightly to p27 will compete for its binding and thus activate Cdk/cyclin complexes. Such small molecules have potential as transient inhibitors of p27-mediated cell cycle arrest and terminal differentiation, and may serve to initiate HC regeneration in the hearing system. We are using fragment-based drug discovery methods, utilizing NMR spectroscopy as the primary screening technique. After the validation of initial fragment hits we used cheminformatics analysis to identify 2nd generation inhibitors. We seek to demonstrate that p27, a prototype IDP, is a ''druggable'' target and that its function can be modulated by small molecules in vivo.

DNA, RNA Structure & Conformation II

3222-Pos Board B83

A New Look at DNA Intercalation is DNA Intercalation Something Artificial or Highly Biologically Relevant?

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Potential roles of intercalative binding have challenged researchers since Lehrman's discovery 50 years ago, that aromatic molecules may reside in between base-pairs. Lately intercalation was found to be used also by bigger systems like operatory proteins. DNA-Recombinases and TATA-box binding protein use intercalative binding for indirect readout of DNA sequences. In such complexes DNA adopts diverse conformations. Using polarized-light spectroscopy and molecular modeling we study DNA complexes with synthetic ruthenium compounds $[(L)_3Ru]^{2+}$ or $[dppz(L)_2Ru]^{2+}$ (L=phenanthroline or bipyridine). We find that depending on level of hydrophobicity, chirality, and size of intercalating moiety DNA indeed adopts different final geometries. With Δ-$[dppz(phenanthroline)_2Ru]^{2+}$ with dppz as intercalating moiety DNA locally converts to an A-like form with characteristic bases roll and sugar-puckering. Not least interesting is DNA bending of 35o induced by partial, ''wedge'', intercalation of phen-ring system of Δ of $[phen_3Ru]^{2+}$ compound. In conclusion, by imitating operatory proteins intercalative binding by small DNA-binding ligands could be used in a variety of ways: for modulating local base and sugar conformation, as well as introducing major changes such as bending in the DNA helix structure.

Fig 1. DNA bends significantly upon ''wedging'' intercalation of $[phen_3Ru]^{2+}$(b), analogously to the observed bending caused by the TATA-box binding protein (a).

3223-Pos Board B84

The Sequence Context and Methylation Dependence of DNA Drug Binding

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The ability of molecules to bind DNA plays an important role in many biological processes including, transcription, regulation, replication, and repair. Understanding the factors that influence binding affinity is therefore essential to our understanding of DNA. In this study the effects of sequence context and binding-site methylation on the binding affinity and conformation of DNA dodecamers are explored. The Cre binding site (ACGT) was studied in its methylated and native forms with a number of different flanking sequences chosen to test the predictive power of a previously published dinucleotide scale, wherein certain dinucleotides were said to have an affinity for either the BI or BII DNA conformation. 31P-NMR was used to test the true conformational state of each phosphorous in the dodecamers, and it was found that the scale could not accurately predict the real conformation of DNA. Importantly, the conformation of the binding site, the central tetrad which was not changed, varied significantly based on the sequence context. Thus more complex relationships than dinucleotides, such as longer-range translational conformation effects, are shown to affect the DNA conformation. Fluorescence titration with the DNA intercalator 7-Aminoactinomycin D was performed to determine the KD of each of the dodecamers and provide biological context to our conformation results. Excellent binding affinities were seen in the ACAC, ACAG, and GGCC sequences, while weaker binding was observed for GGAG sequences. The effects of methylation on binding affinity and conformation demonstrated that methylation effects are modulated by sequence context.

3224-Pos Board B85

Interaction of Porphyrins with tRNA. The Influence of Ionic Strength

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Previously we have investigated the interaction of meso-tetra-(4N-oxyethylpyridyl)porphyrin (TOEPyP4) and its Zn(II), Cu(II), -metallocomplexes with tRNA at low ionic strength [1]. In this work the influence of ionic strength on the interaction of tRNA from E.Coli with TOEPyP4 and its metallocomplexes is studied by UV/Vis Spectrophotometry and Circular Dichroism methods.

The measurements were performed in 0.1 BPSE and 1BPSE buffers (1BPSE = 6 mM Na_2HPO_4+ 2 mM NaH_2PO_4 +185 mM NaCl + 1 mM Na_2EDTA), respectively $\mu = 0.02M$ and $\mu = 0.2M$, pH 6.57.

From the absorption datas the Scatchard binding isotherms for porphyrin-tRNA complexes are built and binding parameters are calculated (N - the number of binding sites per molecule of tRNA, and K - the binding constant).

In case of *low ionic strength* for the values of induced CD spectra (at 400-470 nm) for complexes tRNA with TOEPyP4 and CuTOEPyP4 there is an optimum concentration of porphyrins (0.2-0.25) at which the anisotropy of system is maximal. For complexes of ZnTOEPyP4 with tRNA the induced CD spectra are essentially different. The induced CD spectra of complex change a sign and continue to grow (remaining negative) starting from a certain relative concentration. This unusual ICD spectra profile is found for all three porphyrin-tRNA complexes at *high ionic strength*. It is possible that at high relative concentration of porphyrins the liquid crystal form may exist in the solution.

For all porphyrins the binding constants with tRNA at low ionic strength are an order of magnitude greater than in case of binding at high ionic strength. It means that these porphyrins interact stronger with tRNA at low ionic strength.

Reference

1. Y. Dalyan, I. Vardanyan, A. Chavushyan,G. Balayan. J of Biomol Structure & Dynamics 28, 123-131 (2010).

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DNA Bound Ruthenium Complex Structure Determination by NMR and Optical Spectroscopy

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The design and study of DNA-binding molecules have been of great interest for many years due to their possible applications as diagnostic agents, genetic probes, or chemotherapeutics. Optical spectroscopy methods showed that the binuclear ruthenium complex B (Figure) intercalates into DNA by threading a coordinated ruthenium ion through the DNA base stack, ending up with one subunit in each groove of DNA and the bridging *bidppz* ligand sandwiched between the DNA base pairs. We here investigate by NMR spectroscopy the